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IMMUNOHISTOCHEMICAL EXPRESSION OF GFAP, GS, AQP4, ALZHEIMER-2-ASTROCYTOSIS AND BRAIN AMMONIA LEVELS IN DECEASED SEPTIC PATIENTS WITHOUT LIVER FAILURE AND THOSE WITH SEPSIS-ASSOCIATED LIVER INJURY

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Abstract. Sepsis-associated liver injury (SALI) induces secondary hepatotoxic brain damage, complementing the mechanisms of sepsis-associated encephalopathy. In these conditions, astrocytes play one of the central roles as being the main homeostatic glia and key cells to metabolize ammonia in the brain.

The aim of the study was to determine the ammonia levels and reactive astroglial changes in the brain of deceased septic patients without liver failure and deceased patients with sepsis-associated liver injury.

Materials and methods. Sectional material of 40 patients who died from abdominal sepsis was studied. Case histories were analyzed according to the SOFA scale with accent on the brain and liver disfunction confirmation and excluding kidney insufficiency. Septic cases designed two main comparison groups: 1) sepsis without SALI («non-SALI», n = 20); 2) sepsis with SALI («SALI», n = 20). Control group included autopsy material of 30 deceased patients with cardiovascular pathology with no inflammatory, metabolic or toxic comorbidity. In paraffin sections of the postmortem brain cortex, white matter, hippocampus, thalamus, striatum, and cerebellum, it was determined: I) immunohistochemical expression of GFAP, GS, and AQP4; II) histochemical expression of tissue ammonia with Nessler's reagent according to V. Gutiérrez-de-Juan et al. (2017); III) numbers of Alzheimer type 2 astrocytes (AA2).

Results. In the «non-SALI» group, it is found increased level of all the studied parameters: I) elevated GFAP in six brain regions with the highest growth in the cortex – by 8.46 times; II) elevated GS in the thalamus and cerebellum (by 1.96 and 1.29 times, respectively); III) elevated AQP4 in six brain regions with the highest rise in the cortex – by 3 times; IV) elevated histochemical ammonia expression in the thalamus, striatum, and cerebellum (by 1.29, 1.20, and 1.17 times, respectively); V) increased AA2 numbers in the cortex and thalamus (by 2.32 and 1.53 times, respectively). The «SALI» group is characterized by the decreased GFAP expression in six brain regions, with the lowest values in thalamus, striatum, and cerebellum. Herewith, in six brain regions increased levels are typical for: I) GS expression, with maximal aggravation in the cortex and thalamus (by 3.20 and 3.18 times, respectively); II) AQP4 expression, with maximal increase in thalamus and white matter (by 4.37 and 4.21 times, respectively); III) histochemical ammonia expression with maximal enhancement in thalamus and cerebellum (by 4.33 and 4.27 times, respectively); IV) severity of AA2-astrocytosis with maximal rates in the cortex and striatum (increase by 3.58 and 3.23 times, respectively).

Conclusions. In the brain of deceased septic patients without liver failure, a heterogeneously increased expression of GFAP, AQP4 and GS is observed which is accompanied by a slight increase in the level of tissue ammonia and weak AA2-astrocytosis. In deceased septic patients with sepsis-associated liver injury, a higher level of ammonia in the brain is associated with a significantly reduced level of GFAP, which is accompanied by an enhanced expression of GS and AQP4, as well as more pronounced AA2-astrocytosis, which indicates significant structural and functional remodeling and aggravation of astroglial dystrophy under action of hepatogenic neurotoxicity, which contributes to the disruption of astroglial homeostatic functionality and exacerbates sepsis-associated brain damage.

Keywords: sepsis-associated liver injury, brain, immunohistochemistry, ammonia, Alzheimer type 2 astrocytes.

Introduction. Sepsis-associated liver injury (SALI) is one of the dangerous complications of sepsis and most often develops as a component of multiple organ dysfunction syndrome (MODS), which usually occurs in the later stages of the disease. Clinically, SALI manifests as sepsis-induced cholestasis (jaundice), hypoxic hepatitis, and coagulopathy [1] and significantly increases mortality rates in septic patients [2]. The mechanisms of SALI include the impact of systemic infection, an overactive immune response and microcirculation disorders in the liver, as well as side effects of the main disease therapy [1]. Kupffer cells, hepatocytes, neutrophils and liver sinusoidal endothelial cells are intimately involved in the implementation of noted mechanisms, instigating the synthesis of large amounts of pro-inflammatory cytokines, chemokines, inducing oxidative stress and endothelial dysfunction, finally causing the decline in synthetic, detoxifying and excretory liver functions [1]. Prognostic assessment of sepsis-associated MODS is performed in accordance with the Sequential Organ Failure Assessment (SOFA) scale, where initial hepatic dysfunction is established at the bilirubin level of 1.2–1.9 mg/dl (20-32 μmol/L) [3]. When

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sepsis-associated liver dysfunction progresses to liver failure, it can induce and/or exacerbate a number of other conditions, including: hepatic encephalopathy with cerebral edema, renal and respiratory failure, cardiovascular instability, and coagulopathy. Sepsis-associated encephalopathy (SAE), which develops under these conditions, has a complex mechanism, since in addition to the neurotoxic effect of systemic infection, its decay products, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), it is complicated by a pronounced neurotoxic effect of hyperammonemia, which follows a decrease in the detoxifying capacity of the liver. It is evidenced that during chronic liver failure in liver cirrhosis a morphotypical remodeling astroglia occurs with the appearance of the so-called Alzheimer type 2 astrocytes and changes in the expression of its specific proteins, such as glial fibrillar acidic protein (GFAP), glutamine synthetase (GS) and aquaporin-4 (AQP4) [4, 5]. Also, in patients with chronic and acute liver failure, the level of intracerebral ammonia increases, which was confirmed using neuroimaging methods [6]. Zhao J. and colleagues recently showed the value of plasma ammonia level in incoming septic patients as an independent risk factor for 28-day mortality rate along with the SOFA scale [7]. Furthermore, Numan Y. et al. proposed to use the plasma ammonia level as a newer biomarker of sepsis per se [8]. The morphological changes in astroglia during SAE and hepatotoxic brain damage are only partially described in the literature, which mainly focused on the experimental studies, herewith, to our knowledge, there are no research works describing histochemical determination of the brain ammonia and reactive astroglial transformations in deceased septic patients. Identification of the features of reactive astroglia remodeling under the combined impact of systemic infection and hepatogenic intoxication could provide useful histopathological markers of astroglial dysfunction under these conditions, which could be a useful tool in further scientific research on astrocytic reactivity and elaboration of a novel gliocentric methods to control these processes.

The aim of the study. Determination of ammonia levels and reactive astroglial changes in the brain of deceased septic patients without liver failure and deceased patients with sepsis-associated liver injury.

Materials and methods. The study was performed on autopsy material of 40 patients aged 63±5 years who died in conditions of abdominal sepsis. Clinical and laboratory data from case histories were analyzed according to the SOFA scale [3], providing the criteria for assessing and monitoring organ dysfunction in critically ill patients, namely: respiratory, cardiovascular, hepatic, renal, cerebral (according to the Glasgow coma scale) and the hemostasis systems. Particular attention was focused on a comprehensive assessment of liver dysfunction/failure (analysis of *in vivo* plasma indicators of total bilirubin, AST, ALT, prothrombin index, albumin and post-mortem histopathological examination of the liver) and clinical manifestations of brain dysfunction in accordance with the Glasgow coma scale. Two main comparison groups were designed: 1) sepsis without SALI («non-SALI» group, n = 20); 2) sepsis with SALI («SALI» group, n = 20). Both groups excluded cases of liver cirrhosis, alcoholic and malignant liver pathology, endocrine diseases, chronic/acute renal dysfunction/failure and other chronic toxic-metabolic pathologies. The control group was designed from autopsy material of 30 patients who died from acute cardiovascular insufficiency without concomitant inflammatory, toxic and endocrine pathology. During autopsy, pieces of the cortex and subcortical white matter were taken from the frontal, parietal, temporal, and occipital brain lobes, hippocampus, thalamus, striatum, and cerebellum in the amount that is standard for diagnostic purposes. Brain and liver samples were fixed in 10% buffered formalin followed by standard processing and paraffin embedding. Serial tissue slices of 4 μm thickness were prepared on a precision rotary microtome HM 3600 («MICROM Laborgerate GmbH», Germany); after deparaffination they were stained with hematoxylin and eosin for general histopathology. Immunohistochemistry (IHC) was performed according to the standard protocol provided by the antibody manufacturer using primary antibodies: mouse monoclonal anti-GFAP (clone ASTRO6), rabbit polyclonal anti-GS, rabbit polyclonal anti-AQP4 (all from Thermo Scientific Inc., USA) and visualization system Ultra-Vision Quanto Detection with diaminobenzidine (Thermo Scientific Inc., USA). The IHC reaction was assessed in 5 standardized fields of view (SFV) of each listed brain regions at x200 of the microscope Scope A1 «Carl Zeiss» (Germany) with camera Jenoptik progress Gryphax 60N-C1"1.0x426114 (Germany) and the Videotest-Morphology 5.2.0.158 software (VideoTest LLC). The levels of GFAP, GS and AQP4 expression were evaluated as relative area percentage (Srel., %) of immunopositive labels to the total area of SFV. For estimation of ammonia level in the paraffin brain slices, histochemical (HC) method using Nessler's reagent according to V. Gutiérrez-de-Juan et al. [9] was performed, after which the optical density of HC-positive granules expressed in conditional units of optical density (CUOD) was assessed. The evaluation was processed in each of the selected brain regions, in 5 SFVs of the microscope Scope A1 «Carl Zeiss» (Germany) with Jenoptik progress Gryphax 60N-C1"1.0x426114 (Germany) camera at magnification x200 in automatic regime with standard plugin color deconvolution «H DAB» of ImageJ software. At CUOD values from 0 to 20, the degree of HC ammonia expression in the brain tissue is assessed as negative («-»); from 21 to 50 – as weak («+»); from 51 to 100 – as moderate («++»); from 101 and above – as strong («+++»). In 20 SFVs of each noted brain region, the numbers of Alzheimer type 2 astrocytes (AA2) was counted. This astrocytic morphotype has an enlarged, watery nucleus with punctate chromatin clusters and a noticeable nucleolus adjacent to the nucleolemma, as well as thin rim of the cytoplasm. AA2 nuclei can be at least 2 times the size of the neighboring oligodendrocytes nuclei. According to the numbers of AA2 per 20 SFVs (x400), 4 degrees of AA2-astrocytosis were identified: 1-5 AA2/20 SFVs – 0 degree (absent); 6-10 AA2/20 SFVs – I degree (weak astrocytosis); 11-20 AA2/20 SFVs – II degree (moderate astrocytosis); from 21 and more AA2/20 SFVs – III degree (pronounced astrocytosis).

The data was processed using the Statistica® 13.0 package (StatSoft Inc., License № JPZ804I382130ARCN10-J). Results were expressed as median (Me) with range (Q1; Q3). The Mann-Whitney U-test was used to compare two groups, and the Kruskal-Wallis test was used to compare more than two groups.

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The results were considered statistically significant at the level of 95% ($p < 0.05$).

Results. IHC expression of GFAP, GS, AQP4, as well as HC ammonia expression and AA2 numbers has significant regional heterogeneity in the postmortem brain, both in control group and septic groups.

The IHC expression of GFAP in control reflects the highest values in the subcortical white matter of the cerebral hemispheres and the lowest values in the cerebral cortex (Table 1, Fig. 1). In the «non-SALI» group, there is a significant increase in GFAP expression compared to control values in all the studied brain regions with the maximal increase in the *cerebral cortex* – by 8.46 times and less pronounced increase in the *hippocampus* – by 4.58 times, *white matter* – by 3.10 times, *striatum* – by 2.86 times; *cerebellum* – by 2.80 times and the smallest rise in *thalamus* (by 2.32 times) (Table 1, Fig. 2). The «SALI» group is characterized by significantly ($p < 0.05$) weaker GFAP expression compared to the «SALI» group in all the studied brain regions, herewith only in *cerebral cortex* GFAP expression exceeds the control values ($p < 0.05$) (Table 1). In the postmortem brain from «SALI» group, GFAP expression in the *thalamus*, *striatum,* and *cerebellum* is minimal (Table 1, Fig. 3).

Table 1

GFAP, GS and AQP4 levels (in S rel. (%)), HC ammonia expression (in CUOD), ammonia scale, AA2 numbers and AA2-score in the postmortem brain in «non-SALI», «SALI» and control groups

Brain regions	«non-SALI» group	«SALI» group	Control group
GFAP			
Cerebral cortex	38.26 (27.32; 55.72) *†	10.16 (8.52; 12.49) *†	4.52 (4.23; 5.57)
Subcortical white matter	56.47 (45.24; 64.89) *†	21.32 (15.58; 23.29) †	18.20 (17.11; 18.43)
Hippocampus	32.57 (29.16; 48.23) *†	9.47 (7.34; 11.65) †	7.10 (6.58; 7.89)
Thalamus	14.79 (12.59; 20.45) *†	4.29 (4.11; 6.95) †	6.36 (5.91; 6.79)
Striatum	17.83 (15.45; 22.48) *†	4.48 (4.27; 7.45) †	6.23 (5.70; 7.84)
Cerebellum	15.68 (14.36; 21.34) *†	4.69 (4.36; 6.69) †	5.59 (5.18; 5.83)
GS			
Cerebral cortex	5.37 (4.59; 6.17) †	13.74 (8.72; 14.23) *†	4.29 (2.26; 5.63)
Subcortical white matter	0.59 (0.45; 1.23)	1.12 (0.77; 1.95) *	0.53 (0.34; 0.60)
Hippocampus	2.34 (2.18; 3.49) †	5.92 (4.74; 6.73) *†	2.25 (0.53; 3.90)
Thalamus	4.23 (3.72; 5.06) *†	6.85 (5.58; 7.93) *†	2.15 (1.73; 3.45)
Striatum	2.29 (1.93; 4.47) †	5.72 (5.09; 7.45) *†	1.84 (1.33; 2.12)
Cerebellum	3.15 (2.96; 4.82) *†	7.41 (7.11; 8.52) *†	2.43 (0.63; 2.84)
AQP4			
Cerebral cortex	10.22 (9.79; 11.23) *†	13.93 (12.54; 15.71) *†	3.40 (3.22; 4.25)
Subcortical white matter	3.47 (3.10; 4.96) *†	5.27 (5.07; 6.38) *†	1.25 (0.75; 1.34)
Hippocampus	9.45 (8.83; 10.15) *†	13.26 (12.27; 14.84) *†	4.26 (4.17; 5.25)
Thalamus	3.84 (3.52; 5.93) *†	6.25 (6.17; 7.65) *†	1.43 (0.43; 1.68)
Striatum	4.12 (3.81; 5.58) *†	8.16 (7.23; 9.45) *†	1.95 (1.65; 2.43)
Cerebellum	9.25 (8.28; 10.35) *†	11.58 (11.18; 13.46) *†	3.16 (2.47; 3.75)
HC ammonia expression and Ammonia-scale			
Cerebral cortex	19.11 (12.32; 20.45) †	56.23 (52.56; 65.81) *†	18.14 (15.26; 19.53)
	-	++	-
Subcortical white matter	12.47 (11.15; 16.25) †	21.62 (21.98; 23.64) *†	11.10 (10.34; 14.26)
	-	+	-
Hippocampus	18.12 (15.57; 19.37) †	46.25 (36.45; 47.15) *†	17.25 (14.68; 18.72)
	-	+	-
Thalamus	24.98 (21.43; 25.21) *†	83.47 (68.54; 87.45) *†	19.25 (16.58; 19.72)
	+	++	-
Striatum	22.25 (21.69; 23.60) *†	65.23 (57.38; 74.37) *†	18.46 (15.69; 18.93)
	+	++	-
Cerebellum	23.14 (21.73; 24.81) *†	84.45 (73.36; 85.21) *†	19.74 (18.32; 19.83)
	+	++	-
AA2 numbers and AA2-score			
Cerebral cortex	7.20 (6.50; 8.30) *†	11.10 (10.00; 12.40) *†	3.10 (1.20; 4.50)
	I	II	0

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Subcortical white matter	3.50 (2.90; 5.20) †	7.90 (6.50; 9.70) *†	3.40 (1.80; 4.30)
	0	I	0
Hippocampus	3.20 (1.70; 3.80) †	6.20 (6.10; 9.00) *†	2.70 (1.50; 3.20)
	0	I	0
Thalamus	6.30 (6.00; 9.30) *†	12.60 (11.60; 15.30) *†	4.10 (3.70; 5.20)
	I	II	0
Striatum	4.60 (3.90; 5.80) †	12.30 (11.00; 17.80) *†	3.80 (3.20; 5.90)
	0	II	0
Cerebellum	5.10 (4.10; 5.90) †	11.50 (11.20; 14.20) *†	3.90 (3.80; 5.70)
	0	II	0

Notes: Reliable differences compared to control ($p < 0.05$) are marked with an asterisk (*). Reliable differences between «non-SALI» and «SALI» groups in the same brain region ($p < 0.05$) are marked with the dagger (†).

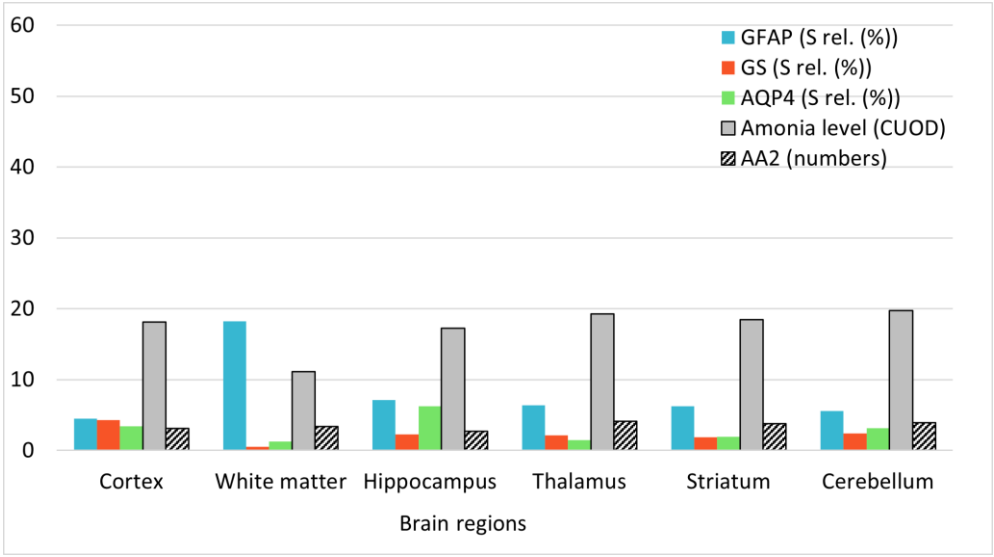

Fig. 1. Medians of GFAP, GS, AQP4 levels, HC ammonia expression and AA2 numbers in different regions of postmortem brain in control group.

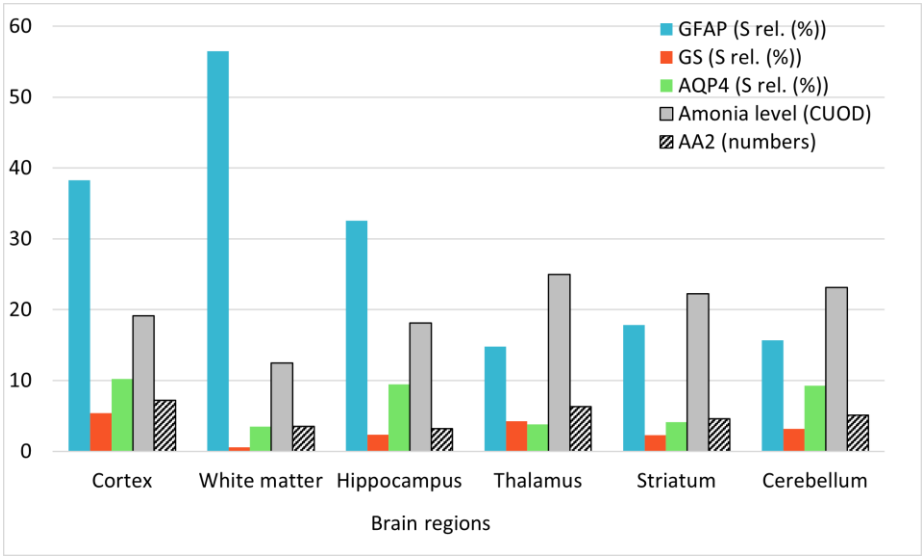

Fig. 2. Medians of GFAP, GS, AQP4 levels, HC ammonia expression and AA2 numbers in different regions of postmortem brain in «non-SALI» group.

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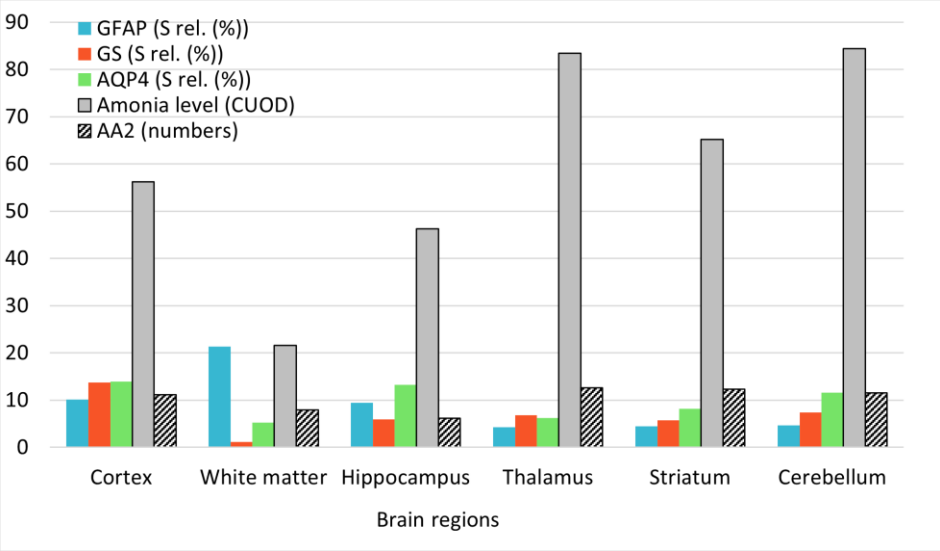

Fig. 3. Medians of GFAP, GS, AQP4 levels, HC ammonia expression and AA2 numbers in different regions of postmortem brain in «SALI» group. Note that the scaling of the vertical axis exceeds the ones in figures 1 and 2.

The IHC expression of GS in control cases is the highest in the cerebral cortex and the lowest in the subcortical white matter (Table 1, Fig. 1). In «non-SALI» group, an increase in GS level compared to control is observed in thalamus and cerebellum (by 1.96 and 1.29 times, respectively) (Table 1, Fig. 2). In «SALI» group, GS expression is significantly ($p < 0.05$) higher compared to «non-SALI» and control values in all studied brain regions, with the exception of the white matter, where the GS expression rates exceed only control ones (Table 1, Fig. 3). In the «SALI» group, there is an increase in GS expression compared to control values: in the *cerebral cortex – by* 3.20 times; *thalamus – by* 3.18 times; *striatum – by* 3.10 times; *cerebellum – by* 3.04 times; *hippocampus – by* 2.63 times and *white matter* – by 2.11 times.

The IHC expression of AQP4 in control group is the highest in the hippocampus and the lowest in the subcortical white matter (Table 1, Fig. 1). In the «non-SALI» group, a significant ($p < 0.05$) increase in AQP4 level compared to control is observed in all 6 studied brain regions: in the *cerebral cortex* – by 3 times; *cerebellum* – by 2.92 times; *white matter* – by 2.77 times, *thalamus* – 2.68 times; *hippocampus* – 2.21 times; and *striatum* – 2.11 times (Table 1, Fig. 2). In the «SALI» group, the expression of AQP4 significantly exceeds the levels of the corresponding brain regions in the «non-SALI» and control groups (Table 1, Fig. 3). Thus, AQP4 expression is higher than control values in the *thalamus* by 4.37 times, in the *white matter* – by 4.21 times, in the *striatum* – by 4.18 times, in the *cerebral cortex* – by 4.09 times, in the *cerebellum* – by 3.66 times, and in the *hippocampus* – by 3.11 times.

The HC ammonia level in control group corresponds to negative expression according to the ammonia scale we used, with relatively higher CUOD values in the cerebellum and the lowest values in the subcortical white matter (Table 1, Fig. 1). In the «non-SALI» group, there is an increase in tissue ammonia level compared to control values in the *thalamus, striatum,* and *cerebellum* (an increase by 1.29, 1.20, and 1.17 times, respectively), which corresponds to weak expression (Table 1, Fig. 2). In the «SALI» group, HC ammonia level significantly ($p < 0.05$) exceeds the values of «non-SALI» and control group in all the studied brain regions (Table 1, Fig. 3). In the «SALI» group, there is an increase in tissue ammonia compared to control in the *thalamus* – by 4.33 times (moderate expression), *cerebellum* – by 4.27 times (moderate expression), *striatum* – by 3.53 times (moderate expression), *cerebral cortex* – by 3.09 times (moderate expression), *hippocampus* – by 2.68 times (weak expression), and *white matter* – by 1.94 times (weak expression).

Alzheimer type 2 astrocytes numbers in control group reflects the absence of AA2-astrocytosis in all studied brain regions (Table 1, Fig. 1). In the «non-SALI» group, there is an increase in AA2 numbers in the *cerebral cortex* and *thalamus* (an increase by 2.32 and 1.53 times, respectively), which corresponds to weak AA2-astrocytosis (Table 1, Fig. 2). In the «SALI» group, AA2 numbers significantly ($p < 0.05$) exceeds the values of the «non-SALI» and control groups in all the brain regions studied (Table 1, Fig. 3). In «SALI» group, AA2 numbers are higher than control indicators: in *cerebral cortex* – by 3.58 times (moderate AA2-astrocytosis), *striatum* – by 3.23 times (moderate AA2-astrocytosis), *thalamus* – by 3.07 times (moderate AA2-astrocytosis), *cerebellum* – by 2.94 times (moderate AA2-astrocytosis), *white matter* – by 2.32 times (weak AA2-astrocytosis), and *hippocampus* – by 2.29 times (weak AA2-astrocytosis).

Discussion. By the results of the present study, we show that during sepsis, astroglia undergo significant morphotypic remodeling induced by exposure to systemic neurotoxins that damage components of the neurogliovascular unit. Astroglia, being the first buffer zone on the way of molecules and cells penetrating the BBB, as well as the main homeostatic cell population in the brain, under action of multifactorial aggressive medium, can undergo dystrophic changes, reducing or modifying its functionality. Experimental studies have evidenced that during systemic and intracerebral inflammation, reactive astrogliosis limits the entry and spread of microbial agents and other aggressive inflammatory factors across the brain, as well as it is involved in the regulation of the local immune response

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during interaction with microglia, and controlling numerous neuronal functions in these issues [10].

One of the first morphological manifestations of reactive astrogliosis is the upregulation of GFAP, one of the intermediate filament proteins expressed predominantly by mature astrocytes, which is associated with the protective responsiveness of astrocytes during various neuropathologies [10]. Postmortem brain of septic patients from the «non-SALI» group is characterized by a significant heterogeneous region-dependent elevation of GFAP level with the highest rates of increase in the cerebral cortex, which indicates more pronounced reactivity of cortical astroglial populations or greater susceptibility of these brain regions to the factors of systemic inflammatory milieu. Water-osmotic imbalance observed in some neuropathologies, is accompanied with decline of GFAP expression followed by structural rearrangements of the cytoskeleton and configuration changes in astrocytes [10]. Our results demonstrate that brains in «SALI» group are characterized by significant drop in GFAP expression compared «non-SALI» group, herewith, GFAP level in the thalamus, striatum and cerebellum does not differ from control levels. This fact indicates that astroglia of the thalamus, striatum and cerebellum are more susceptible to hepatogenic neurotoxicity among the other brain regions studied, undergoing significant structural remodeling in these conditions.

GS, other key functional astrocytic protein, determines the specificity of this cell population for ammonia metabolism in the brain. During sepsis, ammonia plasma level in some patients can be increased even without concomitant liver failure, and its level correlates with the prognosis severity for septic patients [11]. In our study, in «non-SALI» brains there is increase in GS expression in the thalamus and cerebellum, which is accompanied by increased ammonia levels in the same brain regions and indicates the activation of ammonia metabolism in them. Most often, acute liver failure is characterized by high plasma ammonia concentration [12], which causes elevation of the brain ammonia, glutamine overloading of astrocytes and finally their osmotic edema. In the brains of patients from «SALI» group, there is a significant increase in GS expression in all studied brain regions with the maximal growth in the cortex, thalamus, striatum and cerebellum, which may indicate regional heterogeneity in the ammonia metabolism, as well as neurotransmitters associated with the latter. The ammonia level in the brain of septic patients with SALI demonstrates the most pronounced increase in the thalamus, cerebellum and striatum, which reflects the same trend as the heterogeneous expression of GS. It is noteworthy that the predominant regionality of tissue ammonia elevation in thalamus, striatum, and cerebellum among the same six brain regions was described in our recent study of postmortem brain of cirrhotic patients, where ammonia level correlated with the severity of liver cirrhosis [13].

Pathophysiology of the brain edema in SAE are largely associated with hyperexpression of AQP4, the main water channel protein in the brain, primarily localized in the vascular astrocytic end feet. In this study, we demonstrate that postmortem brains of septic patients without SALI are characterized by heterogeneous increase in AQP4 expression with the highest elevation in the cerebral cortex and cerebellum, which is generally consistent with results of our previous septic study in rodents [14]. During liver failure, overexpression of AQP4 plays a multifactorial role in ammonia-mediated osmotic edema of astrocytes and subsequent generalized brain edema [15]. SALI development in septic patients is accompanied by an even greater increase in brain AQP4 expression compared to «non-SALI» group, with the maximal rates in thalamus.

Alzheimer type 2 astrocytes, being neuropathological hallmark of hepatic encephalopathy, can occur in single quantities during some critical conditions accompanied by acute ion-osmotic imbalance [4]. Septic brains from the «non-SALI» group demonstrate weak AA2 astrocytosis found in the cortex and thalamus, highlighting these anatomical brain regions or their astroglial populations as the most sensitive to ammonia concentrations and other possible actors of systemic and local aggressive milieu. The development of SALI is accompanied by more numerous AA2 with the maximal appearance in the cortex, striatum, thalamus and cerebellum, which coincides with the regional distribution of the maximal levels of tissue ammonia, GS and AQP4 and the minimal levels of GFAP. The regional combination of the greatest decrease in GFAP expression with the highest degree of AA2-astrocytosis indirectly confirms the notion that AA2 are characterized by a reduced or completely absent expression of GFAP, leading to substantial conformational changes in the astrocytic cytoskeleton [16], therefore, a significant accumulation of AA2, as well as their morphotypical precursors, leads to a general decline of GFAP levels in the brain regions with established necessary conditions for these reactive astroglial changes.

Conclusions:

1. The postmortem brains of septic patients with no liver failure are characterized by increased expression of GFAP and AQP4 in the cortex, subcortical white matter, hippocampus, thalamus, striatum and cerebellum, increased expression of GS in thalamus and cerebellum; a slight elevation of tissue ammonia in thalamus, striatum, and cerebellum, and weak AA2-astrocytosis in the cerebral cortex and thalamus.
2. In the postmortem brains of septic patients with sepsis-associated liver injury, all studied brain regions demonstrate markedly decreased GFAP expression (most reduced in thalamus, striatum, and cerebellum), herewith increased expression of GS and AQP4, gain in brain tissue ammonia, as well as mild and moderate AA2-astrocytosis (compared to control and septic group without liver failure).

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ІММУНОГІСТОХІМІЧНА ЕКСПРЕСІЯ GFAP, GS, AQP4, АЛЬЦГЕЙМЕР-2-АСТРОЦИТОЗ І РІВЕНЬ АМІАКУ В МОЗКУ ПОМЕРЛИХ ХВОРИХ НА СЕПСИС БЕЗ ПЕЧІНКОВОЇ НЕДОСТАТНОСТІ І ПОМЕРЛИХ З СЕПСИС-АСОЦІЙОВАНИМ ПОШКОДЖЕННЯМ ПЕЧІНКИ

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Резюме. Сепсис-асоційоване пошкодження печінки (САПП) зумовлює вторинне гепатотоксичне ушкодження головного мозку (ГМ), доповнюючи механізми сепсис-асоційованої енцефалопатії.

Мета. Визначення рівня аміаку та реактивних змін астроглії в ГМ померлих септичних хворих без печінкової недостатності та померлих хворих з сепсис-асоційованим пошкодженням печінки.

Матеріали і методи. Досліджено секційний матеріал 40 хворих, які померли від абдомінального сепсису. Виділено 2 групи спостереження: 1) сепсис без САПП («бСАПП», n = 20); 2) сепсис із САПП («САПП», n = 20). Імуногістохімічно визначали експресію GFAP, GS і AQP4, гістохімічно – експресію тканинного аміаку з реактивом Несслера і підраховували кількість астроцитів Альцгеймера 2 типу (АА2) у корі та білій речовині ГМ, гіпокампі, таламусі, смугастому тілі та мозочку.

Результати. У групі «бСАПП» підвищується експресія GFAP та AQP4 у 6 відділах ГМ, GS – *у таламусі та мозочку*; експресія аміаку – в *таламусі, смугастому тілі* та *мозочку*; кількість АА2 підвищується у *корі* та *таламусі*. У групі «САПП» у 6 відділах ГМ відзначається падіння експресії GFAP при одночасному підвищенні рівнів: GS (максимально – в корі і таламусі), AQP4 (максимально – в *таламусі* і *білій речовині),* аміаку (максимально – в *таламусі* та *мозочку)* та вираженості АА2-астроцитозу (максимально – в *корі* та *смугастому тілі*).

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Висновки. У померлих хворих на сепсис без печінкової недостатності в ГМ підвищена експресія GFAP, AQP4 та GS, відзначається слабке підвищення рівня тканинного аміаку і слабкий АА2-астроцитоз. У померлих з САПП більш високий рівень аміаку в ГМ асоційований зі значно зниженим рівнем експресії GFAP, підвищеною експресією GS і AQP4 і більш вираженим АА2-астроцитозом, що вказує на істотне структурно-функціональне ремоделювання астроглії.

Ключові слова: сепсис-асоційоване пошкодження печінки, головний мозок, імуногістохімія, аміак, астроцити Альцгеймера 2 типу.

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